PATENT
Attorney Docket No. AVIGEN-04082

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Couto *et al.*
Serial No.: 09/470,618 Group No.: 1633
Filed: 12/22/99 Examiner: Nikodem, D.
Entitled: **Adeno-Associated Vectors for Expression of Factor VIII by Target Cells**

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF HAND DELIVERY

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being hand delivered in an envelope addressed to **David Nikodem**.

Dated: OCT. 26, 2000 By: Jane Edwards
Jane Edwards

Declaration Submitted Under 37 C.F.R. 1.132

Examiner Nikodem:

I, Linda Couto, hereby declare as follows:

1. I am employed by Avigen, Inc., an Assignee of record in the above-referenced application, where I hold the title of Director of Hematology and direct research and development activities relating to recombinant adeno-associated virus (rAAV)-based gene delivery systems for treating hemophilia, as well as other blood disorders and diseases.

2. I hold a Bachelor of Science (B.S.) degree in Biology from Northeastern University and a Doctorate (Ph.D) in Molecular Toxicology, from the Massachusetts Institute of Technology. I have conducted research in the fields of DNA mutagenesis/repair, virology, and gene therapy. As a post-doctoral fellow at Stanford University, I studied the molecular basis of DNA repair in mammalian cells and lower eukaryotes. For the past 10 years, I have worked in the gene therapy field using both retroviral and adeno-associated viral vectors to treat hemophilia. In my position as Director of Hematology at Avigen, I direct research

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in hemophilia gene therapy using adeno-associated viral based technologies, and have co-authored many scientific publications on this topic.

3. I am a co-inventor of U.S. Patent Application Serial No. 09/470,618; the '618 application). I understand that the Specification has been objected to as failing to provide an enabling disclosure. In particular, the Examiner argues that Applicant has not provided evidence for the correlation for the mouse model shown and the use of the same construct to treat hemophilia in humans.

4. It is my opinion that the '618 application provides sufficient disclosure to enable those of ordinary skill in the art to practice the claimed invention. My opinion is based on the facts set forth below, as well as my familiarity with the subject matter.

5. In particular, my co-workers and I, as well as a number of other researchers who practice in the field, have shown successful *in vivo* delivery of rAAV vectors containing various therapeutic genes, as well as sustained expression of these various genes after delivery, and therapeutic benefit in treated animals including preliminary indications of therapeutic benefit in humans. These results demonstrate the broad applicability of the methodology claimed in the '618 application.

6. In the manuscript attached hereto at Tab A, my co-workers describe a study in which sustained, long-term treatment of both moderate and severe -thalassemia was attained using a single administration of rAAV vector containing murine EPO gene. Two different mouse model systems were used in the study, a first strain (T/T) having a naturally occurring genetic defect in -globin (Skow et al. (1983) Cell 34:1043; Popp et al. (1985) Ann. NY Acad. Sci. 445: 432; attached hereto at Tabs B and C, respectively), and a second strain (T/N) of transgenic thalassemic mice (Paszty, C. (1997) Curr. Opin. Hematol. 4:88; attached hereto at Tab D). Both of these animal model systems are art-recognized models for studying thalassemia. The vectors were administered to the T/T and T/N subjects using a single intramuscular (IM) injection. Hematocrit, blood hemoglobin, reticulocyte and serum EPO levels were assayed over the course of the studies (ranging from 4 to 6 months). Spleen and heart weights were determined at the time of necropsy. Histopathological analyses of tissue and spleen were also performed. In the data

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reported in our manuscript at Tab A, elevations in hematocrit and hemoglobin were sustained over the course of the study in both animal models. Modest increases in red blood cell volume and deformability were also seen. Heart weights were significantly reduced in both groups of treated animals, and body weight and splenic health were closer to normal in the treated animals relative to control animals. In short, a therapeutic benefit in both moderate and severe - thalassemic mice was observed.

7. James Wilson and co-workers (Fisher *et al.* (1997) Nature Med. 3:306; attached hereto at Tab E), and Paul D. Kessler and co-workers (Kessler *et al.* (1996) Proc. Nat. Acad. Sci. 93:14082; attached hereto at Tab F), have described stable gene transfer and subsequent expression of a reporter gene in mice following *in vivo* administration of rAAV vector. In the study, rAAV vector containing the *LacZ* reporter gene was administered by direct IM injection into mice. The authors report that high efficiency gene transfer was observed, with most of the muscle fibers in the area of injection staining positive for the transferred LacZ marker protein. The same methods were used in primates (rhesus monkeys), and the authors report that about 20 percent of the muscle fibers in a $25mm^2$ area at the site of injection stained positive for the presence of reporter protein.

8. Katherine A. High and co-workers (Herzog *et al.* (1997) Proc. Nat. Acad. Sci. USA 94:5804; attached hereto at Tab G) have describe stable gene transfer and expression of therapeutic levels of human Factor IX via a single, IM administration of rAAV vector. In particular, the authors drew upon previous studies that showed persistent expression of reporter genes in muscle fibers of animals transduced with rAAV vector (Herzog *et al.*, at page 5807, first column). Based on the successes obtained with the reporter genes, the authors prepared a rAAV vector containing the human Factor IX (hF.IX) gene and administered the it to 4 different strains of mice by direct injection into the hindlimbs (1×10^{10} or about 2×10^{11} vector genomes per animal, for a total of 150 μl/animal). Expression of the hF.IX transgene was assayed using immunofluorescence staining of tissue sections and ELISA of plasma samples. In the results reported (Herzog *et al.*, at page 5807, second column), therapeutic levels of human Factor IX in the circulation of treated animals were attained (*i.e.*, 200-350 ng/ml). This expression was stable for at least 6 months. Based on these findings, the authors conclude administration of rAAV vectors expressing hF.IX to patients with

hemophilia B should be a feasible strategy for treatment of the disease. (Herzog *et al.*, at See page 5809, second column).

9. Richard Snyder and co-workers (Snyder *et al.* (1997) Nature Genetics 16:270; attached hereto at Tab H) and Nakai and co-workers (Nakai *et al.* (1998) Blood 91:4600; attached hereto at Tab I) describe studies where a persistent and therapeutic concentration of Factor IX was achieved in mice after hepatic gene transfer using rAAV vectors. The rAAV vector was administered to murine subjects via a single portal vein infusion in doses ranging from about 2×10^{10} to 8×10^{10} particles. Serum samples were taken, and human Factor IX determination by ELISA was conducted. These authors detected human Factor IX within a week of treatment, and persistent expression, ranging from 250-2,500 ng/ml, was attained within several weeks and lasted throughout the study (36 weeks) (Snyder *et al.*, at page 270, second column). Three additional studies were then conducted to demonstrate appropriate biological activity of the human Factor IX protein found in the treated animal's serum. The authors report that the human Factor IX protein in the mouse serum exhibited Factor IX clotting activity (Snyder *et al.*, at page 271-273). Based on these results, the authors conclude that rAAV gene delivery systems can be used to achieve persistent and therapeutic concentrations of human Factor IX.

10. I am a co-author of the publication attached hereto at Tab J (Kay *et al.* (2000) Nature Gen. 24:257), which describes expression of human Factor IX after low-dose IM administration of rAAV vector in three adult human subjects with severe hemophilia B. Before treatment, the study patients experienced spontaneous bleeding events 2-3 times per week, particularly in joints and soft tissue. Evidence for gene transfer and expression after vector administration was measured by muscle biopsy, by measuring circulating Factor IX levels, and by assessing the number of bleeding episodes and the frequency of clotting factor infusion. Muscle biopsies were performed 8-12 weeks after vector administration, and PCR on DNA extracted from injected muscle was positive for vector sequences in all three patients. Immunohistochemical staining of skeletal muscle was positive for F.IX in the extracellular space, a pattern that had been documented in pre-clinical studies for Factor IX secreted by muscle fibers. Additional sections analyzed by routine histology showed no evidence of inflammation or muscle injury. Patient #1, who had a baseline Factor IX level of

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less than 1% before treatment, peaked at a 1.6%, and now has between 0.5% and 0.8% Factor IX activity levels. Furthermore, this patient experienced a 50-60% reduction in the number of bleeding episodes. Patient # 2 now has 0.8% activity and has experienced a 90% reduction in bleeding episodes. The third patient having the mild-severe form of hemophilia B showed no change in clinical status or Factor IX levels. Consequently, this study demonstrates that Factor IX expression activity in murine animal model systems described in Herzog *et al.* (Tab G) is predictive of activity in humans.

11. In light of the information provided above in paragraph 4-10, Applicants submit that the murine data disclosed in the application enables use of the invention in humans. The data reported by my co-workers and numerous others skilled in the field of rAAV gene delivery systems, collectively demonstrate that the methods described and claimed in the '618 application are currently being practiced by a number of researchers, and based on their widely reported successes, such methods do not require undue experimentation. Researchers working with rAAV gene delivery systems consistently use *in vivo* mouse models to assess both transduction and expression efficiency of genes (*See*, Exhibits A-G). The methods described and claimed in the '618 application are currently being practiced by a number of researchers and, based on their widely reported successes, such methods do not require undue experimentation. Furthermore, studies conducted in murine animal model systems with AAV are art-recognized as reliable predictors of activity in other animals. That the murine animal model is a reliable predictor of activity in humans is further shown by the data reported in Exhibit H.

12. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 10/24/00 By: Linda B. Couto

Linda Couto, Ph.D.

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